

	PCR 20 µL reaction (units : µL)	Final Concentration		Primers:
5X Phusion HF Buffer	4	1X		f_Gbs_yeGFP
10 mM dNTPs	0,4	200 µM		r_Gbs_yeGFP
10 µM Forward Primer	1	0.5 µM		
10 µM Reverse Primer	1	0.5 µM		
CMV-Ds-Red2 diluted (5ng/µL)	1	5 ng		DNA Template:
DMSO (optional)	0,6	3 %		pCYC_yeGFP
Phusion DNA Polymerase (add last)	0,2	1.0 units/50 µl PCR		concentration: 825.4 ng/µl
Nuclease-free Water	11,8			--> Take 1 µl in 824.4 µl of Water
Total volume	20			> New concentration 1 ng/µl
Number of reactions (including controls):	4			
WORK ON ICE				
Mastermix	5 reactions needed			
5X Phusion HF Buffer	16			
10 mM dNTPs	1,6			
10 µM Forward primer	4			
10 µM Reverse primer	4			
DMSO (optional)	2,4			
Phusion DNA Polymerase (ADDED LAST)	0,8			
Nuclease-free Water	51,2			
Distribute 19 µl in tubes				
Programme A : 'Y-pCYC-A'				
Initial Denaturation	98°C	30 seconds		
	98°C	10 seconds		
	55°C	20 seconds		
35 Cycles	72°C	180 seconds		
Final Extension	72°C	10 minutes		
Hold	4°C			
Programme B : 'Y-pCYC-B'				
Initial Denaturation	98°C	30 seconds		
	98°C	10 seconds		
	55°C	20 seconds		
35 Cycles	72°C	160 seconds		
Final Extension	72°C	10 minutes		
Hold	4°C			